TEXT SEARCHABLE DOCUMENT - 2009

Data Evaluation Record on the Acute Toxicity of 4775453 (M07; Metabolite of BAS 800 H) to Aquatic Vascular Plants, *Lemna gibba*

PMRA Submission Number: 2008-0431
PMRA Document ID: 1664720 EPA MRID Number: 47560302

Data Requirement:		
	PMRA DATA CODE	9.8.5
	EPA DP Barcode	349851
	OECD Data Point	IIA 8.6
	EPA MRID	47560302
	EPA Guideline	OPPTS 850.4400 (123-2)

Test material: 4775453 (M07; metabolite of BAS 800 H) **Purity:** 95.4%
Common name: Saflufenacil metabolite
Chemical name: IUPAC: N-{4-chloro-2-fluoro-5-[({[isopropyl(methyl)amino]sulfonyl}amino)carbonyl]phenyl}-N′-methylurea
CAS name: Not reported
CAS No.: Not reported
Synonyms: Not reported

Moncie V Wright

Primary Reviewer: Moncie Wright **Signature:**
Staff Scientist, Cambridge Environmental **Date:** 11/25/08

Secondary Reviewer: Teri S. Myers **Signature:** Teri S Myers
Senior Scientist, Cambridge Environmental **Date:** 12/01/08

Primary Reviewer: Anita Pease **Date:** 06/09/09 APease 6/9/09
Senior Biologist, U.S. EPA

Secondary Reviewer: Ann Lee **Date:** 06/09/09
PMRA/APVMA

Secondary Reviewer: Farzad Jahromi **Date:** 06/09/09
DEWHA-APVMA

Company Code	BAZ
Active Code	SFF
Use Site Category:	13 (terrestrial feed crops) and 14 (terrestrial food crops)
EPA PC Code	118203

CITATION: Porch, J.R., Kendall, T.Z., Krueger, H.O., and C. Holmes. 2008. BAS 800 H Metabolite M07: A 7-Day Toxicity Test with Duckweed (*Lemna gibba* G3). Unpublished study performed by Wildlife International, Ltd., Easton, MD. Wildlife International Study No.: 147A-243. Study sponsored by BASF Corporation, Research Triangle Park, North Carolina. BASF Study No.: 355549. Study completed August 28, 2008.

DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute toxicity of a pesticide to aquatic vascular plants. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data

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requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

EXECUTIVE SUMMARY:

In a 7-day acute toxicity study, the freshwater floating aquatic vascular plants Duckweed (*Lemna gibba*) were exposed to 4775453 (M07; metabolite of BAS 800 H) at nominal concentrations of 0 (negative control), 3.9, 6.5, 11, 18, and 30 mg a.i./L under static conditions. Mean-measured concentrations were <2.10 (<LOQ, control), 3.8, 6.3, 11, 18, and 30 mg a.i./L.

No endpoint was sensitive to treatment, as growth was promoted for all endpoints, relative to the negative control group.

A small percentage (≤0.41%) of necrotic and/or chlorotic fronds was observed at all treatment levels and in the control at study termination.

This toxicity study is classified as **ACCEPTABLE** to the **U.S. EPA** and as **FULLY RELIABLE** to **PMRA and APVMA** as it is scientifically sound and satisfies the guideline requirement for a Tier II vascular plant toxicity study with the freshwater species, *Lemna gibba*.

Results Synopsis

Test Organism: *Lemna gibba*
Test Type (Flow-through, Static, Static Renewal): Static

Frond density
EC_{05}: >30 mg a.i./L 95% C.I.: N/A
EC_{50}: >30 mg a.i./L 95% C.I.: N/A
NOAEC: 30 mg a.i./L
Probit Slope: N/A

Growth rate (based on frond number)
EC_{05}: >30 mg a..i./L 95% C.I.: N/A
EC_{50}: >30 mg a.i./L 95% C.I.: N/A
NOAEC: 30 mg a.i./L
Probit Slope: N/A

Biomass (Dry weight)
EC_{05}: >30 mg a.i./L 95% C.I.: N/A
EC_{50}: >30 mg a.i./L 95% C.I.: N/A
NOAEC: 30 mg a.i./L
Probit Slope: N/A

Endpoint(s) Affected: none

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: This study was conducted following EPA Series 850 - Ecological Effects

Test Guidelines OPPTS Number 850.4400, ASTM Standard Guide 1415-91 E : *Standard Guide for Conducting Static Toxicity Tests with Lemna gibba* G3 (1991), and OECD Guideline 221: *Lemna sp. Growth Inhibition Test.* The following deviations from OPPTS 850.4400 were noted:

1. The physicochemical properties of the test material were not reported.
2. Pretest health of the test species was not reported.
3. At test initiation the pH of the test solutions ranged from 7.6 to 8.0, and at study termination the pH ranged from 8.9 to 9.0, above the pH suggested by OPPTS guidelines of 7.5.
4. This test was conducted without a renewal; OPPTS guidelines suggest one renewal for a 7-day test with *Lemna* spp. However, recovery of the test material at study termination indicated that it was stable under the test conditions.
5. Initial biomass was estimated using a sample in triplicate of the inoculum culture rather than a sample of the inoculum culture.
6. Chemical analysis of the samples was performed using an analytical method that was not validated.

These deviations do not affect the acceptability of the study.

COMPLIANCE: Signed and dated No Data Confidentiality, GLP, Quality Assurance, and Certification statements were provided. This study was conducted in compliance with U.S. EPA FIFRA GLP standards (40 CFR Part 160 and 792; 1998), OECD Principles of GLP and JMAFF GLP (1999), with the following exception:
Periodic well water screening analyses for potential contaminants were not performed according to Good Laboratory Practice Standards, but were performed using a certified laboratory and standard U.S. EPA analytical methods.

A. MATERIALS:

1. Test material 4775453 (M07; metabolite of BAS 800 H)

Description: Solid.

Lot No./Batch No. : L67-196 (Batch no.)

Purity: 95.4%

Stability of compound under test conditions: The day 7 mean-measured concentrations yielded recoveries of *ca.* 93 to 97% of the nominal test concentrations, indicating that 4775453 was stable under the test conditions.

(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)

Storage conditions of test chemicals: Test material was stored under ambient conditions.

Physicochemical properties of 4775453.

Parameter	Values	Comments
Water solubility at 20°C	**Not reported.**	
Vapor pressure	**Not reported.**	
UV absorption	**Not reported.**	
pKa	**Not reported.**	
Kow	**Not reported.**	

2. Test organism:

Name: Duckweed (*Lemna gibba*) *EPA requires a vascular species: Lemna gibba.*
Strain, if provided: G3
Source: In-house cultures originally obtained from the USDA.
Age of inoculum: At least 2 weeks
Method of cultivation: Grown under test conditions (20X-AAP)

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: A range-finding study was conducted with a negative control, and 0.49, 1.6, 5.4, 18, and 60 mg/L treatment groups. % inhibition in frond number in the treatment groups when compared to the negative control were 10, 21, 1, -4, and 8%. Fronds appeared normal in the control and first three treatment groups, and there was frond curl and one necrotic frond in the two highest treatment levels.

b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks
		Criteria
Acclimation period:	Continuous.	
Culturing media and conditions: (same as test or not)	Same as test.	
Health: (any mortality observed)	Not reported.	
Test system		

Parameter	Details	Remarks *Criteria*
Static/static renewal Renewal rate for static renewal	Static N/A	*EPA expects the test concentrations to be renewed every 3 to 4 days (one renewal for the 7 day test, 3-4 renewals for the 14 day test).*
Incubation facility	Test vessels were placed in a temperature-controlled environmental chamber.	
Duration of the test	7 days	*EPA requires a duration of 14 days. Seven day studies will be accepted for review by the Agency.*
Test vessel Material: *(glass/stainless steel)* Size: Fill volume:	 Glass 250 mL 100 mL	

Parameter	Details	Remarks *Criteria*
Details of growth medium name pH at test initiation: pH at test termination: Chelator used: Carbon source:	 7.6-8.0 8.9-9.0 Yes $NaHCO_3$	*EPA recommends the following culture media: Modified Hoagland's E+ or 20X-AAP. Chelating agents (e.g. EDTA) are recommended in the nutrient medium for optimum cell growth. Lower concentrations of chelating agents (down to one-third of the normal concentration recommended for AAP medium) may be used in the nutrient medium used for test solution preparation if it is suspected that the chelator will interact with the test material. ASTM reference, E1415-91and D 3978-80 (reapproved 1987).*
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	Yes	
Dilution water source/type: pH: water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	 Purified well water Adjusted to 7.5 ± 0.1. Filter-sterilized. Not reported. Not reported. See Reviewer's Comments. None Detected. Not reported.	*EPA recommends a pH of ~5.0. A solution pH of 7.5 is acceptable if type 20X-AAP nutrient media is used.*
Indicate how the test material is added to the medium (added directly or used stock solution)	A stock solution was prepared at the highest test concentration, and was serially diluted to obtain the lower test concentrations.	
Aeration or agitation	Neither.	

Parameter	Details	Remarks *Criteria*
Sediment used **(for rooted aquatic vascular plants)** Origin: Textural classification (%sand, silt, and clay): Organic carbon (%): Geographic location:	N/A	
Number of replicates Control: Solvent control: Treatments:	3 N/A 3	
Number of plants/replicate	Not reported.	*EPA requires 5 plants.*
Number of fronds/plant	12 fronds per replicate	*EPA requires 3 fronds per plant.*
Test concentrations Nominal: Measured:	0 (negative control), 3.9, 6.5, 11, 18, and 30 mg a.i./L <2.10 (<LOQ, control), 3.8, 6.3, 11, 18, and 30 mg a.i./L	*EPA requires at least 5 test concentrations with a dose range of 2X or 3X progression.*
Solvent (type, percentage, if used)	N/A	
Method and interval of analytical verification	All exposure solutions, calibration standards, and matrix fortification samples were analyzed using HPLC with UV detection (220 nm). Test solutions were analyzed at test initiation and termination.	

Parameter	Details	Remarks *Criteria*
Test conditions Temperature: Photoperiod: Light intensity and quality:	 23.8-26.6°C Continuous. 4450 to 4800 lux Warm-white fluorescent lighting	
Reference chemical (if used) name: concentrations:	N/A	
Other parameters, if any	None.	

2. Observations:

Table 2: Observation parameters

Parameters	Details	Remarks/Criteria
Parameters measured (e.g.,: number of fronds, plant dry weight or other toxicity symptoms)	Number of fronds, growth rate (based on frond number and biomass), and biomass	Observations of effects such as chlorosis, necrosis, dead fronds, root destruction, and break-up of duckweed colonies were also performed.
Measurement technique for frond number and other end points	Visual counts were used for frond density. Dry weight (biomass) was determined by drying fronds for 2 days and then weighing. Growth rate was determined based on frond density and biomass.	
Observation intervals	Days 0, 3, 5, and 7.	
Other observations, if any	See Inhibitory Effects.	
Indicate whether there was an exponential growth in the control	Yes. Frond density was 153 fronds/replicate in the negative control at test termination.	
Were raw data included?	For frond density and biomass, but not for growth rate.	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

By test termination, frond densities averaged 153 fronds/rep in the negative control, yielding inhibitions of -3.3, -10, -4.4, -14, and -5.4% when compared to the negative control in the mean-measured 3.8, 6.3, 11, 18, and 30 mg a.i./L treatment groups, respectively. Based on frond density, the study author's NOAEC and EC_{50} values were 30 and >30 mg a.i./L, respectively.

Growth rates based on frond number were 0.364 $days^{-1}$ in the negative control, yielding inhibitions of -1.2, -3.7, -1.6, -5.1, and -2.1% when compared to the negative control. Based on growth rate, the NOAEC and EC_{50} values were 30 and >30 mg a.i/L, respectively.

Biomass (dry weight) averaged 19.0 mg in the negative control, yielding inhibitions of -4.6, -8.6, -5.6, -17, and -1.4% when compared to the negative control. Based on biomass, the NOAEC and EC_{50} values were 30 and >30 mg a.i/L, respectively.

The study authors also analyzed growth rate based on biomass, which resulted in an average growth rate of 0.399 $days^{-1}$ in the negative control, yielding inhibitions of -1.5, -2.7, -1.8, -5.2, and -0.47% when compared to the negative control. Based on biomass growth rate, the NOAEC and EC_{50} values were 30 and >30 mg a.i/L, respectively.

The study authors used mean-measured concentrations for calculations of endpoints, and compared frond density, growth rate, and biomass treatment groups to the negative control.

A small percentage (not exceeding 0.41%) of necrotic and/or chlorotic fronds was observed at all treatment levels and the control at study termination.

Table 3: Effect of 4775453 on frond number of Duckweed, *Lemna gibba*

Mean-measured and (Nominal) Concentrations (mg a.i./L)	Initial frond number/test solution	frond number at			
		3 days	5 days	7 days	
				frond number	% inhibition
Negative control	12	36	82	153	N/A
3.8 (3.9)	12	36	83	158	-3.3
6.3 (6.5)	12	35	85	169	-10
11 (11)	12	35	83	160	-4.4
18 (18)	12	37	88	175	-14
30 (30)	12	34	83	162	-5.4
Reference chemical (if used)	N/A	N/A	N/A	N/A	N/A

Table 4: Effect of 4775453 on growth of Duckweed, *Lemna gibba*

Mean-Measured and (Nominal) Concentrations mg ai/L	Initial frond number/test solution	Frond Number Growth rate (days^{-1}, mean)	Frond Number Growth rate % Inhibition	Biomass, dry weight (mg, mean)	Biomass % Inhibition
Negative control	12	0.364	N/A	19.0	N/A
3.8 (3.9)	12	0.368	-1.2	19.8	-4.6
6.3 (6.5)	12	0.377	-3.7	20.6	-8.6
11 (11)	12	0.369	-1.6	20.0	-5.6
18 (18)	12	0.382	-5.1	22.2	-17
30 (30)	12	0.372	-2.1	19.2	-1.4
Reference chemical (if used)	N/A	N/A	N/A	N/A	N/A

Table 5: Statistical endpoint values.

Statistical Endpoint	Frond No.	Frond number growth rate/biomass growth rate	Biomass
NOAEC or EC_{05} (mg a.i./L)	30	30	30
LOAEC (mg a.i./L)	N/A	N/A	N/A
IC_{50} or EC_{50} (mg a.i./L) (95% C.I.)	>30 (N/A)	>30 (N/A)	>30 (N/A)
Other (IC_{25}/EC_{25})	N/A	N/A	N/A
Reference chemical NOAEC IC_{50}/EC_{50}	N/A	N/A	N/A

B. REPORTED STATISTICS:

Day 7 EC_{50} values were determined using linear interpolation with treatment response and exposure concentration data. The data was tested for normality using Shapiro-Wilks' Test, and for homogeneity of variance using Levene's Test ($\alpha = 0.05$). The treatment group data were compared to the control using ANOVA and Dunnett's test. The NOAEC was determined using the results of the statistical analyses and an evaluation of the dose-response pattern. All statistical analyses were conducted using Toxstat Version 3.5.

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Growth was promoted at all treatment levels; therefore, statistical analysis was not conducted.

Frond density
EC_{05}: >30 mg a..i./L 95% C.I.: N/A
EC_{50}: >30 mg a.i./L 95% C.I.: N/A
NOAEC: 30 mg a.i./L
Probit Slope: N/A

Growth rate (based on frond number)
EC_{05}: >30 mg a..i./L 95% C.I.: N/A
EC_{50}: >30 mg a.i./L 95% C.I.: N/A
NOAEC: 30 mg a.i./L
Probit Slope: N/A

Biomass (Dry weight)
EC_{05}: >30 mg a..i./L 95% C.I.: N/A
EC_{50}: >30 mg a.i./L 95% C.I.: N/A
NOAEC: 30 mg a.i./L
Probit Slope: N/A

D. STUDY DEFICIENCIES:

This test was conducted without a renewal; OPPTS guidelines suggest one renewal for a 7-day test with *Lemna* spp. However, recovery of the test material at study termination indicated that it was stable under the test conditions.

E. REVIEWER'S COMMENTS:

The reviewer's and the study authors' results were in agreement.

Results from the periodic screening analysis of the dilution water indicated the presence of the following components: calcium (38.7 mg/L), chloride (4.2 mg/L), fluoride (0.55 mg/L), magnesium (14.6 mg/L), potassium (6.97 mg/L), sodium (19.8 mg/L) and sulfate (6.0 mg/L).

The test solutions appeared clear and colorless.

The in-life portion of the test was conducted from August 5 to 12, 2008.

F. CONCLUSIONS:

Indicate if the study is scientifically sound and is classified as ACCEPTABLE to the U.S. EPA and as FULLY RELIABLE to PMRA and APVMA. The most sensitive endpoint could not be determined, as all endpoints were unaffected by the test material.

Frond density
EC_{05}: >30 mg a..i./L 95% C.I.: N/A
EC_{50}: >30 mg a.i./L 95% C.I.: N/A
NOAEC: 30 mg a.i./L
Probit Slope: N/A

Growth rate (based on frond number)
EC_{05}: >30 mg a..i./L 95% C.I.: N/A
EC_{50}: >30 mg a.i./L 95% C.I.: N/A
NOAEC: 30 mg a.i./L
Probit Slope: N/A

Biomass (Dry weight)
EC_{05}: >30 mg a..i./L 95% C.I.: N/A
EC_{50}: >30 mg a.i./L 95% C.I.: N/A
NOAEC: 30 mg a.i./L
Probit Slope: N/A

Endpoint(s) Affected: none

III. REFERENCES:

1. U.S. Environmental Protection Agency. 1996. Series 850 - Ecological Effects Test Guidelines (Draft), OPPTS Number 850.4400: *Aquatic Plant Toxicity Test using Lemna sp., Tiers I and II.* Washington, D.C.

2. ASTM Standard Guide 1415-91E. 1991. *Standard Guide for Conducting Static Toxicity Tests with Lemna gibba G3*. American Society for Testing and Materials. Philadelphia, PA.
3. Organization for Economic Cooperation and Development. 2006. OECD Guidelines for the Testing of Chemicals. Guideline 221: *Lemna sp. Growth Inhibition Test.*
4. Microsoft Corporation. Microsoft Excel 2000. Copyright 1985-1989.
5. West, Inc. and D.D. Gulley. 1996. TOXSTAT® Version 3.5. Western Ecosystems Technology, Inc. Cheyenne, Wyoming.

Data Evaluation Report on the Acute Toxicity of BAS 781 02 H (formulation containing 54.6% Dimethenamid-P and 6.2% Saflufenacil) to Aquatic Vascular Plants, *Lemna gibba*

PMRA Submission Number: 2008-0432
PMRA Document ID: 1662899 EPA MRID Number: 47560404

Data Requirement:		
	PMRA DATA CODE	9.8.6
	EPA DP Barcode	349851
	OECD Data Point	IIIA 10.8.2.1
	EPA MRID	47560404
	EPA Guideline	OPPTS 850.4400 (123-2)

Test material: BAS 781 02 H (AI: Saflufenacil) **Purity:** 54.6% (BAS 656 H; Dimethenamid-P) and 6.2% (BAS 800H)
Common name: Dimethenamid-P formulation
Chemical name: IUPAC: BAS 656 H: (S)-2-chloro-N-(2,4-dimethyl-3-thienyl)-N-(2-methoxy-1-methylethyl)acetamide; not reported for a.i. Saflufenacil
CAS name: Not Reported
CAS No.: BAS 656 H: 163515-14-8; not reported for a.i. Saflufenacil

Moncie V Wright

Primary Reviewer: Moncie Wright **Signature:**
Staff Scientist, Cambridge Environmental **Date:** 11/11/08

Secondary Reviewer: Teri S. Myers **Signature:** Teri S Myers
Senior Scientist, Cambridge Environmental **Date:** 11/18/08

Primary Reviewer: Anita Pease **Date:** 06/09/09 Apease 6/9/09
Senior Biologist, U.S. EPA

Secondary Reviewer: Ann Lee **Date:** 06/09/09
HC-PMRA-EAD

Secondary Reviewer: Farzad Jahromi **Date:** 06/09/09
DEWHA-APVMA

Company Code	BAZ
Active Code	SFF
Use Site Category:	13 (terrestrial feed crops) and 14 (terrestrial food crops)
EPA PC Code	118203

CITATION: Minderhout, T., Kendall, T.Z., Krueger, H.O., and C. Holmes. 2008. BAS 781 02 H: A 7-Day Toxicity Test with Duckweed (*Lemna gibba* G3). Unpublished study performed by Wildlife International, Easton, MD. Laboratory Project ID: Wildlife International Study No. 147A-241. Study sponsored by BASF Corporation, Research Triangle Park, North Carolina. BASF Study No.: 355547. Study completed August 28, 2008.

DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute toxicity of a pesticide to aquatic vascular plants. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data

requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

EXECUTIVE SUMMARY:

In a 7-day acute toxicity study, the freshwater floating aquatic vascular plants Duckweed (*Lemna gibba*) were exposed to BAS 781 02 H (formulation containing 54.6% Dimethenamid-p and 6.2% Saflufenacil) at nominal concentrations of 0 (negative control), 0.41, 1.2, 3.7, 11, 33, and 100 µg form/L under static conditions. Only concentrations of dimethenamid-p were analytically verified. Given the stability of dimethenamid-p and the lack of analytical verification for the formulation, all endpoints are reported in terms of nominal concentrations.

The 7-Day NOAEC and EC_{50} values for biomass, the most sensitive endpoint, were 1.2 and 23 µg BAS 781 02 H/L, respectively. The % growth inhibition in biomass in treatment levels, as compared to the control, ranged from 5.7 to 65%.

At test termination, there was severe necrosis and limited chlorosis and mortality in the highest three treatment levels.

It can be concluded that both dimethenamid-p and saflufenacil contribute to the toxicity of BAS 781 based on comparison of the range of results of *Lemna* 14d EC_{50} values for technical dimethenamid-p (8.9 to 13 µg/L; e-Pesticide Manual, MRID 44332257) and the 7d EC_{50} value for technical saflufenacil of 85 µg/L (IIA 8.6; MRID 47127922; PMRA 1547234).

This toxicity study is classified as **ACCEPTABLE** to the **U.S. EPA** and as **FULLY RELIABLE** to **PMRA and APVMA** as it is scientifically sound and satisfies the guideline requirement for a Tier II vascular plant toxicity study with the freshwater species, *Lemna gibba.*

Results Synopsis

Test Organism: *Lemna gibba*
Test Type (Flow-through, Static, Static Renewal): Static

BAS 781 02 H
Frond density

EC_{05}:	0.59 µg form/L	95% C.I.: 0.25 to 1.4 µg form/L
EC_{50}:	28 µg form/L	95% C.I.: 22 to 37 µg form/L
NOAEC:	1.2 µg form/L	
Probit Slope:	0.979 ± 0.0805	

Growth rate

EC_{05}:	2.1 µg form/L	95% C.I.: 1.2 to 3.7 µg form/L
EC_{50}:	100 µg form/L	95% C.I.: 87 to 120 µg form/L
NOAEC:	1.2 µg form/L	
Probit Slope:	0.977 ± 0.0688	

Biomass (Dry weight)

EC_{05}:	0.27 µg form/L	95% C.I.: 0.058 to 1.2 µg form/L

EC_{50}: 23 µg form/L 95% C.I.: 14 to 37 µg form/L
NOAEC: 1.2 µg form/L
Probit Slope: 0.854 ± 0.109

Endpoint(s) Affected: frond density, growth rate, and biomass (most sensitive)

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: This study was conducted following EPA Series 850 - Ecological Effects Test Guidelines OPPTS Number 850.4400, ASTM Standard Guide 1415-91 E (1991), and OECD Guideline 221: *Lemna sp. Growth Inhibition Test.* The following deviations from OPPTS 850.4400 were noted:

1. The physicochemical properties of the test material were not reported.
2. Pretest health of the test species was not reported.
3. At test initiation and termination, the pH of the solutions was 7.8 to 8.0 and 8.6 to 8.9, respectively, well above the pH suggested by OPPTS guidelines of 7.5.
4. A static test with no renewals was conducted. At least one renewal is recommended for studies conducted with Lemna species. However, concentrations appeared to be stable over the study period.

These deviations do not affect the study acceptability.

COMPLIANCE: Signed and dated No Data Confidentiality, GLP, Quality Assurance, and Certification statements were provided. This study was conducted in compliance with U.S. EPA FIFRA GLP standards (40 CFR Part 160 and 792; 1989), OECD Principles of GLP and JMAFF GLP (1999), with the following exception:

Periodic well water screening analyses for potential contaminants were not performed according to Good Laboratory Practice Standards, but were performed using a certified laboratory and standard U.S. EPA analytical methods.

A. MATERIALS:

1. Test material BAS 781 02 H (formulation containing 54.6% Dimethenamid-P and 6.2% Saflufenacil)

Description: Liquid

Lot No./Batch No. : 1632-78 (Batch No.)

Purity: 54.6% BAS 656 H and 6.2% BAS 800 H

Stability of compound under test conditions: The day 0 measured concentrations yielded recoveries of 84.8 to 130% of

nominal, while day 7 concentrations yielded recoveries of 79.1 to 146% of nominal, indicating that BAS 781 02 H was stable under the test conditions.

(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)

Storage conditions of test chemicals: Test material was stored under ambient conditions.

Physicochemical properties of BAS 781 02 H (AI: Saflufenacil).

Parameter	Values	Comments
Water solubility at 20°C	Not reported.	
Vapor pressure	Not reported.	
UV absorption	Not reported.	
pKa	Not reported.	
Kow	Not reported.	

2. Test organism:

Name: Duckweed (*Lemna gibba*) *EPA requires a vascular species: Lemna gibba.*
Strain, if provided: G3
Source: In-house cultures originally obtained from the USDA.
Age of inoculum: At least 2 weeks
Method of cultivation: Grown under test conditions (20X-AAP)

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: A range-finding study was conducted with nominal test concentrations of 1.4, 4.5, 8.1, 15, 27, 90, 300, and 1000 µg/L, with percent inhibitions of 1.6, 28, 44, 45, 62, 77, 81, and 90% as compared to the negative control.

b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation period: Culturing media and conditions: (same as test or not) Health: (any mortality observed)	Continuous. Temperature and photoperiod appeared to be the same as test conditions. Not reported.	
Test system Static/static renewal Renewal rate for static renewal	Static N/A	*EPA expects the test concentrations to be renewed every 3 to 4 days (one renewal for the 7 day test, 3-4 renewals for the 14 day test).*
Incubation facility	Test vessels were placed in a temperature-controlled environmental chamber.	
Duration of the test	7 days	*EPA requires a duration of 14 days. Seven day studies will be accepted for review by the Agency.*
Test vessel Material: *(glass/stainless steel)* Size: Fill volume:	Glass 250 mL 100 mL	

Parameter	Details	Remarks *Criteria*
Details of growth medium name pH at test initiation: pH at test termination: Chelator used: Carbon source:	 7.8-8.0 8.6-8.9 Yes $NaHCO_3$	*EPA recommends the following culture media: Modified Hoagland's E+ or 20X-AAP. Chelating agents (e.g. EDTA) are recommended in the nutrient medium for optimum cell growth. Lower concentrations of chelating agents (down to one-third of the normal concentration recommended for AAP medium) may be used in the nutrient medium used for test solution preparation if it is suspected that the chelator will interact with the test material. ASTM reference, E1415-91and D 3978-80 (reapproved 1987).*
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	Yes	
Dilution water source/type: pH: water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	 Purified well water Adjusted to 7.5. Filter-sterilized. Not reported. Not reported. See Reviewer's Comments None Detected. Not reported.	*EPA recommends a pH of ~5.0. A solution pH of 7.5 is acceptable if type 20X-AAP nutrient media is used.*
Indicate how the test material is added to the medium (added directly or used stock solution)	A secondary stock solution was prepared using a primary stock solution to obtain a nominal concentration of 100 µg/L. This secondary stock solution was then serially diluted with freshwater algal medium to obtain the remaining lower test concentrations.	

Parameter	Details	Remarks *Criteria*
Aeration or agitation	Neither.	
Sediment used (**for rooted aquatic vascular plants**) Origin: Textural classification (%sand, silt, and clay): Organic carbon (%): Geographic location:	N/A	
Number of replicates Control: Solvent control: Treatments:	 3 N/A 3	
Number of plants/replicate	4 plants	*EPA requires 5 plants.*
Number of fronds/plant	3 fronds per plant	*EPA requires 3 fronds per plant.*
Test concentrations Nominal:	0 (negative control), 0.41, 1.2, 3.7, 11, 33, and 100 µg form/L	Only concentrations of dimethenamid-p were analytically verified. Given the stability of dimethenamid-p and the lack of analytical verification for the formulation, all endpoints are reported in terms of nominal concentrations. *EPA requires at least 5 test concentrations with a dose range of 2X or 3X progression.*
Solvent (type, percentage, if used)	N/A	

Parameter	Details	Remarks *Criteria*
Method and interval of analytical verification	All samples, calibration standards, and matrix fortification samples were analyzed using HPLC with UV detection (240 nm). Test solutions were analyzed at test initiation and termination.	
Test conditions Temperature: Photoperiod: Light intensity and quality:	 23.8-26.6°C Continuous. 4320 to 5390 lux Warm-white fluorescent lighting	
Reference chemical (if used) name: concentrations:	N/A	
Other parameters, if any	None.	

2. Observations:

Table 2: Observation parameters

Parameters	Details	Remarks/Criteria
Parameters measured (e.g.,: number of fronds, plant dry weight or other toxicity symptoms)	Number of fronds, growth rate (based on frond number and biomass), and biomass	Observations of effects such as chlorosis, necrosis, dead fronds, root destruction, and break-up of duckweed colonies were also performed.
Measurement technique for frond number and other end points	Visual counts were used for frond density. Dry weight (biomass) was determined by drying fronds for 2 days and then weighing. Growth rate was determined based on frond number and biomass.	
Observation intervals	Days 0, 3, 5, and 7.	
Other observations, if any	See Inhibitory Effects.	
Indicate whether there was an exponential growth in the control	Yes. Frond density was 131 fronds/replicate in the negative control at test termination.	

Parameters	Details	Remarks/Criteria
Were raw data included?	Yes.	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

After 96 hours of exposure, frond number averaged 131 fronds/replicate in the negative control, yielding inhibitions of 5.1, 2.5, 20, 36, 52, and 70% as compared to the negative control at the mean-measured 0.57, 1.1, 3.5, 10, 28, and 91 µg/L treatment levels, respectively. The 7-day NOAEC and EC_{50} values based on frond number were reported by the study authors to be 1.1 and 26 µg/L, respectively.

Biomass averaged 18.6 mg in the negative control, yielding inhibitions of 5.7, 2.0, 19, 41, 62, and 65% as compared to the negative control. The 7-day NOAEC and EC_{50} values based on biomass were reported by the study authors to be 1.1 and 18 µg/L, respectively.

Growth rate based on frond number averaged 0.342 $days^{-1}$ in the negative control, yielding inhibitions of 2.3, 1.1, 9.6, 19, 30, and 50% as compared to the negative control. The 7-day NOAEC and EC_{50} values based on growth rate were reported by the study authors to be 1.1 and 90 µg/L, respectively.

Growth rate based on biomass averaged 0.420 $days^{-1}$ in the negative control, yielding inhibitions of 1.9, 0.66, 6.7, 17, 30, and 32% as compared to the negative control. The 7-day NOAEC and EC_{50} values based on growth rate were reported by the study authors to be 1.1 and >91 µg/L, respectively.

At test termination, there was severe necrosis and limited chlorosis and mortality in the highest three treatment levels.

Table 3: Effect of BAS 781 02 H (AI: Saflufenacil) on frond number of Duckweed, *Lemna gibba*

Mean-measured and (Nominal) Concentrations (µg/L)	Initial frond number/test solution	frond number at			
		3 days	5 days	7 days	
				frond number	% inhibition
Negative control	12	39	73	131	N/A
0.57 (0.41)	12	36	73	125	5.1
1.1 (1.2)	12	35	73	128	2.5
3.5 (3.7)	12	37	67	105	20
10 (11)	12	33	62	84	36
28 (33)	12	28	43	64	52
91 (100)	12	23	32	39	70

Reference chemical (if used)	N/A	N/A	N/A	N/A	N/A

Table 4: Effect of BAS 781 02 H (AI: Saflufenacil) on growth of Duckweed, *Lemna gibba*

Mean-Measured and (Nominal) Concentrations (µg/L)	Initial frond number/test solution	Growth rate (days $^{-1}$, mean)	Growth rate % Inhibition	Biomass, dry weight (mg, mean)	Biomass % Inhibition
Negative control	12	0.342	N/A	18.6	N/A
0.57 (0.41)	12	0.334	2.3	17.5	5.7
1.1 (1.2)	12	0.338	1.1	18.2	2.0
3.5 (3.7)	12	0.309	9.6	15.0	19
10 (11)	12	0.279	19	10.9	41
28 (33)	12	0.238	30	7.1	62
91 (100)	12	0.170	50	6.5	65
Reference chemical (if used)	N/A	N/A	N/A	N/A	N/A

N/A- not applicable

Table 5: Statistical endpoint values (Expressed in terms of BAS 781 02 H)*.

Statistical Endpoint	Frond No.	Growth rate (based on frond number)	Biomass
NOAEC or EC_{05} (µg/L)	1.1	1.1	1.1
LOAEC (µg/L)	3.5	3.5	3.5
IC_{50} or EC_{50} (µg/L) (95% C.I.)	26 (17-42)	90 (80-92)	18 (10-24)
Other (IC_{25}/EC_{25})	N/A	N/A	N/A
Reference chemical NOAEC IC_{50}/EC_{50}	N/A	N/A	N/A

*Study author-reported values.

B. REPORTED STATISTICS:

Statistical analysis was performed for the endpoints of cell density, biomass (area under the growth curve), and

growth rate using Toxstat Version 3.5. Linear interpolation was used to calculate EC_{50} values and their corresponding confidence intervals when possible. The data was tested for normality using Shapiro-Wilks' Test, and for homogeneity of variance using Levene's Test. Treatment groups were compared to the control using ANOVA and Dunnett's t-test. The results of the statistical analyses and an evaluation of the concentration-response pattern were used to determine the NOAEC and LOAEC values.

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The reviewer tested the data for normality using the Chi-square and Shapiro Wilks tests and homogeneity of variance using the Hartley and Bartlett's test. If the data met the assumptions of ANOVA, the NOAEC values were determined using the parametric Williams' and Dunnett's tests. If the data did not meet the assumptions of ANOVA, the NOAEC values were determined using the non-parametric Kruskal-Wallis test and visual interpretation of the data. The ECx values and probit slopes were determined using the probit analysis. All analyses were conducted using the nominal concentrations and Nuthatch statistical software. Values input for growth rate were multiplied by 1000 to eliminate means with a zero value.

BAS 781 02 H

Frond density

EC_{05}: 0.59 µg form/L 95% C.I.: 0.25 to 1.4 µg form/L
EC_{50}: 28 µg form/L 95% C.I.: 22 to 37 µg form/L
NOAEC: 1.2 µg form/L
Probit Slope: 0.979 ± 0.0805

Growth rate

EC_{05}: 2.1 µg form/L 95% C.I.: 1.2 to 3.7 µg form/L
EC_{50}: 100 µg form/L 95% C.I.: 87 to 120 µg form/L
NOAEC: 1.2 µg form/L
Probit Slope: 0.977 ± 0.0688

Biomass (Dry weight)

EC_{05}: 0.27 µg form/L 95% C.I.: 0.058 to 1.2 µg form/L
EC_{50}: 23 µg form/L 95% C.I.: 14 to 37 µg form/L
NOAEC: 1.2 µg form/L
Probit Slope: 0.854 ± 0.109

D. STUDY DEFICIENCIES:

There were no study deficiencies.

E. REVIEWER'S COMMENTS:

The reviewer and the registrant's results were similar. However, the reviewer's results are based on nominal concentrations of the formulated product, as discussed below. The reviewer's results are presented in the Executive Summary and Conclusions sections of this DER.

Concentrations of Saflufenacil (BAS 800H) were not measured in this study. The analytical determination was conducted for the primary active ingredient, Dimethenamid-P, only. Analytically verified concentrations of

dimethenamid-p were 85-103% of nominal on day 0. Although the stability of saflufenacil was not measured under test conditions, it is reasonable to expect dissolved saflufenacil to decline based on the results of the *Lemna* study conducted with technical saflufenacil (IIA 8.6; MRID-47127922; PMRA 1547234). In addition, saflufenacil is susceptible to hydrolysis at this pH (IIA 2.9.1; MRID-47127823; PMRA-1546926). Considering the high recovery and stability of dimethenamid-p and the lack of measured concentrations for the whole formulation, all biological endpoints are reported in terms of nominal concentrations.

Results from the periodic screening analysis of the dilution water indicated the presence of the following components: calcium (38.7 mg/L), chloride (4.2 mg/L), fluoride (0.55 mg/L), magnesium (14.6 mg/L), potassium (6.97 mg/L), sodium (19.8 mg/L) and sulfate (6.0 mg/L).

After mixing by inversion, the test solutions appeared clear and colorless.

The in-life portion of the test was conducted from August 6 to 13, 2008, and the dry weight measurements were completed on August 15, 2008.

F. CONCLUSIONS:

The study is scientifically sound and is classified as ACCEPTABLE to the U.S. EPA and as FULLY RELIABLE to PMRA and APVMA. The 7-Day NOAEC and EC_{50} values for biomass, the most sensitive endpoint, were 1.2 and 23 µg BAS 781 02 H/L, respectively.

BAS 781 02 H
Frond density
EC_{05}: 0.59 µg form/L 95% C.I.: 0.25 to 1.4 µg form/L
EC_{50}: 28 µg form/L 95% C.I.: 22 to 37 µg form/L
NOAEC: 1.2 µg form/L
Probit Slope: 0.979 ± 0.0805

Growth rate
EC_{05}: 2.1 µg form/L 95% C.I.: 1.2 to 3.7 µg form/L
EC_{50}: 100 µg form/L 95% C.I.: 87 to 120 µg form/L
NOAEC: 1.2 µg form/L
Probit Slope: 0.977 ± 0.0688

Biomass (Dry weight)
EC_{05}: 0.27 µg form/L 95% C.I.: 0.058 to 1.2 µg form/L
EC_{50}: 23 µg form/L 95% C.I.: 14 to 37 µg form/L
NOAEC: 1.2 µg form/L
Probit Slope: 0.854 ± 0.109

Endpoint(s) Affected: frond density, growth rate, and biomass (most sensitive)

III. REFERENCES:

1. U.S. Environmental Protection Agency. 1996. Series 850 - Ecological Effects Test Guidelines (Draft), OPPTS Number 850.4400: *Aquatic Plant Toxicity Test using Lemna spp., Tiers I and II.* Washington, D.C.Yan, Z.

1998. AC 375839: Determination of Solubility in Water Using the Shake Flask Method BASF Reg. Doc. Number BN-311-001.

2. Organization for Economic Cooperation and Development. Working Draft of a Proposal for a New Guideline 221A *Lemna sp. Growth Inhibition Test*. Circulated 9 April 2004.
3. ASTM Standard Guide 1415-91E. 1991. *Standard Guide for Conducting Static toxicity tests with Lemna gibba G3*. American Society for Testing and Materials. Philadelphia, PA.
4. Schulz, H., and M. Meyer. 2007. *Determination of Dimethenamid-P and Its Metabolites M23 and M27 in Tap and Surface Water – Validation of the Method 519/0*. SGS Institut Fresenius GmbH Project Number IF-07/00871632. BASF DocID 2007/1054384.
5. Microsoft Corporation. Microsoft Excel 2000. Copyright 1985-1989.
6. West, Inc. and D.D. Gulley. 1996. TOXSTAT® Version 3.5. Western Ecosystems Technology, Inc. Cheyenne, Wyoming.
7. Norberg-King, T.J. 1993. *A Linear Interpolation Method for Sublethal Toxicity: The Inhibition Concentration (ICp) Approach*. Version 2.0. U.S. EPA. National Effluent Toxicity Center. Duluth, Minnesota. Technical Report 03-93.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

```
BAS 781 02 H & L. gibba 7-day frond number ug/L
File: 0404f       Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies
--------------------------------------------------------------------------------

INTERVAL     <-1.5     -1.5 to <-0.5     -0.5 to 0.5     >0.5 to 1.5     >1.5
             _____     _____________     ___________     ___________     _____

EXPECTED     1.407          5.082             8.022          5.082       1.407
OBSERVED     0              8                 6              7           0

--------------------------------------------------------------------------------
Calculated Chi-Square goodness of fit test statistic =   5.7230
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.


BAS 781 02 H & L. gibba 7-day frond number ug/L
File: 0404f       Transform: NO TRANSFORMATION

Shapiro Wilks test for normality
--------------------------------------------------------------------------------

D =  666.000

W =    0.978

Critical W (P = 0.05) (n = 21) = 0.908
Critical W (P = 0.01) (n = 21) = 0.873
--------------------------------------------------------------------------------

Data PASS normality test at P=0.01 level. Continue analysis.


BAS 781 02 H & L. gibba 7-day frond number ug/L
File: 0404f       Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance
--------------------------------------------------------------------------------

Calculated H statistic (max Var/min Var) =   49.00
Closest, conservative, Table H statistic = 1705.0  (alpha = 0.01)

Used for Table H ==>     R (# groups) =   7,    df (# reps-1) =       2
Actual values    ==>     R (# groups) =   7,    df (# avg reps-1) =   2.00

--------------------------------------------------------------------------------

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal
      but do not differ greatly, the Hartley test may still be used
      as an approximate test (average df are used).
```

BAS 781 02 H & L. gibba 7-day frond number ug/L
File: 0404f Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance

Calculated B statistic = 8.58
Table Chi-square value = 16.81 (alpha = 0.01)
Table Chi-square value = 12.59 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 2.00
Used for Chi-square table value ==> df (#groups-1) = 6

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

BAS 781 02 H & L. gibba 7-day frond number ug/L
File: 0404f Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	22679.143	3779.857	79.457
Within (Error)	14	666.000	47.571	
Total	20	23345.143		

Critical F value = 2.85 (0.05,6,14)
Since F > Critical F REJECT Ho:All groups equal

BAS 781 02 H & L. gibba 7-day frond number ug/L
File: 0404f Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	Neg control	131.333	131.333		
2	0.41	124.667	124.667	1.184	
3	1.2	128.000	128.000	0.592	
4	3.7	104.667	104.667	4.735	*
5	11	84.333	84.333	8.346	*
6	33	63.667	63.667	12.016	*
7	100	39.333	39.333	16.337	*

Dunnett table value = 2.53 (1 Tailed Value, P=0.05, df=14,6)

BAS 781 02 H & L. gibba 7-day frond number ug/L
File: 0404f Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	Neg control	3			
2	0.41	3	14.248	10.8	6.667
3	1.2	3	14.248	10.8	3.333
4	3.7	3	14.248	10.8	26.667
5	11	3	14.248	10.8	47.000
6	33	3	14.248	10.8	67.667
7	100	3	14.248	10.8	92.000

BAS 781 02 H & L. gibba 7-day frond number ug/L
File: 0404f Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Neg control	3	131.333	131.333	131.333
2	0.41	3	124.667	124.667	126.333
3	1.2	3	128.000	128.000	126.333
4	3.7	3	104.667	104.667	104.667
5	11	3	84.333	84.333	84.333
6	33	3	63.667	63.667	63.667
7	100	3	39.333	39.333	39.333

BAS 781 02 H & L. gibba 7-day frond number ug/L
File: 0404f Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Neg control	131.333				
0.41	126.333	0.888		1.76	k= 1, v=14
1.2	126.333	0.888		1.85	k= 2, v=14
3.7	104.667	4.735	*	1.88	k= 3, v=14
11	84.333	8.346	*	1.89	k= 4, v=14
33	63.667	12.016	*	1.90	k= 5, v=14
100	39.333	16.337	*	1.91	k= 6, v=14

s = 6.897
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	0.59	0.25	1.4	0.17	0.43
EC10	1.4	0.69	2.8	0.14	0.50
EC25	5.8	3.6	9.3	0.098	0.62
EC50	28.	22.	37.	0.055	0.77

Slope = 0.979 Std.Err. = 0.0805

Goodness of fit: p = 0.38 based on DF= 4.0 14.

LEMNAF~1.TXT : BAS 781 02 H & L. gibba 7-day frond number ug/L

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	3.00	131.	132.	-1.06	100.	0.00
0.410	3.00	125.	128.	-2.97	96.4	3.60
1.20	3.00	128.	121.	7.47	91.0	8.96
3.70	3.00	105.	107.	-2.08	80.6	19.4
11.0	3.00	84.3	86.8	-2.50	65.6	34.4
33.0	3.00	63.7	62.7	0.946	47.4	52.6
100.	3.00	39.3	39.1	0.201	29.6	70.4

BAS 781 02 H & L. gibba 7-day growth rate (ug/L)
File: 0404g Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.407	5.082	8.022	5.082	1.407
OBSERVED	0	8	6	7	0

Calculated Chi-Square goodness of fit test statistic = 5.7230
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

BAS 781 02 H & L. gibba 7-day growth rate (ug/L)
File: 0404g Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 1295.333

W = 0.968

```
Critical W (P = 0.05) (n = 21) = 0.908
Critical W (P = 0.01) (n = 21) = 0.873
--------------------------------------------------------------------------------

Data PASS normality test at P=0.01 level. Continue analysis.


BAS 781 02 H & L. gibba 7-day growth rate (ug/L)
File: 0404g         Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance
--------------------------------------------------------------------------------

Calculated H statistic (max Var/min Var) =   17.45
Closest, conservative, Table H statistic = 1705.0  (alpha = 0.01)

Used for Table H ==>     R (# groups) =   7,    df (# reps-1) =       2
Actual values    ==>     R (# groups) =   7,    df (# avg reps-1) =   2.00

--------------------------------------------------------------------------------

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal
      but do not differ greatly, the Hartley test may still be used
      as an approximate test (average df are used).


BAS 781 02 H & L. gibba 7-day growth rate (ug/L)
File: 0404g         Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance
--------------------------------------------------------------------------------

Calculated B statistic =    5.43
Table Chi-square value =   16.81  (alpha = 0.01)
Table Chi-square value =   12.59  (alpha = 0.05)

Average df used in calculation  ==>   df (avg n - 1) =   2.00
Used for Chi-square table value ==>   df (#groups-1) =   6
--------------------------------------------------------------------------------

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is
      used to calculate the B statistic (see above).


 BAS 781 02 H & L. gibba 7-day growth rate (ug/L)
File: 0404g         Transform: NO TRANSFORMATION

                                ANOVA TABLE
--------------------------------------------------------------------------------

SOURCE             DF             SS                MS             F
--------------------------------------------------------------------------------
Between             6          73556.476         12259.413        132.500
```

Within (Error)	14	1295.333	92.524
Total	20	74851.810	

Critical F value = 2.85 (0.05,6,14)
Since F > Critical F REJECT Ho:All groups equal

BAS 781 02 H & L. gibba 7-day growth rate (ug/L)
File: 0404g Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	Neg control	341.667	341.667		
2	0.41	334.333	334.333	0.934	
3	1.2	338.000	338.000	0.467	
4	3.7	309.000	309.000	4.159	*
5	11	278.667	278.667	8.022	*
6	33	238.333	238.333	13.157	*
7	100	169.667	169.667	21.900	*

Dunnett table value = 2.53 (1 Tailed Value, P=0.05, df=14,6)

BAS 781 02 H & L. gibba 7-day growth rate (ug/L)
File: 0404g Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	Neg control	3			
2	0.41	3	19.870	5.8	7.333
3	1.2	3	19.870	5.8	3.667
4	3.7	3	19.870	5.8	32.667
5	11	3	19.870	5.8	63.000
6	33	3	19.870	5.8	103.333
7	100	3	19.870	5.8	172.000

BAS 781 02 H & L. gibba 7-day growth rate (ug/L)
File: 0404g Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Neg control	3	341.667	341.667	341.667
2	0.41	3	334.333	334.333	336.167
3	1.2	3	338.000	338.000	336.167

4	3.7	3	309.000	309.000	309.000
5	11	3	278.667	278.667	278.667
6	33	3	238.333	238.333	238.333
7	100	3	169.667	169.667	169.667

BAS 781 02 H & L. gibba 7-day growth rate (ug/L)
File: 0404g Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Neg control	341.667				
0.41	336.167	0.700		1.76	k= 1, v=14
1.2	336.167	0.700		1.85	k= 2, v=14
3.7	309.000	4.159	*	1.88	k= 3, v=14
11	278.667	8.022	*	1.89	k= 4, v=14
33	238.333	13.157	*	1.90	k= 5, v=14
100	169.667	21.900	*	1.91	k= 6, v=14

s = 9.619
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	Upper	Std.Err.	Lower Bound /Estimate
EC5	2.1	1.2	3.7	0.12	0.57
EC10	5.0	3.2	7.7	0.090	0.65
EC25	21.	16.	27.	0.051	0.78
EC50	1.0E+02	87.	1.2E+02	0.032	0.86

Slope = 0.977 Std.Err. = 0.0688

Goodness of fit: p = 0.44 based on DF= 4.0 14.

LEMNAG~1.TXT : BAS 781 02 H & L. gibba 7-day growth rate (ug/L)

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	3.00	342.	341.	0.678	100.	0.00
0.410	3.00	334.	338.	-3.35	99.0	0.968
1.20	3.00	338.	331.	7.19	97.0	2.99
3.70	3.00	309.	314.	-4.72	92.0	8.00
11.0	3.00	279.	282.	-3.39	82.7	17.3
33.0	3.00	238.	233.	5.33	68.3	31.7
100.	3.00	170.	171.	-1.74	50.3	49.7

!!!Warning: EC50 not bracketed by doses evaluated.

```
BAS 781 02 H & L. gibba 7-day biomass (ug/L
File: 0404b       Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies
--------------------------------------------------------------------------------

INTERVAL     <-1.5     -1.5 to <-0.5     -0.5 to 0.5     >0.5 to 1.5     >1.5
             -----     -------------     -----------     -----------     ----

EXPECTED     1.407         5.082             8.022           5.082       1.407
OBSERVED     0             7                 7               7           0

--------------------------------------------------------------------------------
Calculated Chi-Square goodness of fit test statistic =   4.3919
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.


BAS 781 02 H & L. gibba 7-day biomass (ug/L
File: 0404b       Transform: NO TRANSFORMATION

Shapiro Wilks test for normality
--------------------------------------------------------------------------------

D =   17.200

W =    0.983

Critical W (P = 0.05) (n = 21) = 0.908
Critical W (P = 0.01) (n = 21) = 0.873
--------------------------------------------------------------------------------

Data PASS normality test at P=0.01 level. Continue analysis.


BAS 781 02 H & L. gibba 7-day biomass (ug/L
File: 0404b        Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance
--------------------------------------------------------------------------------

Calculated H statistic (max Var/min Var) =   36.21
Closest, conservative, Table H statistic = 1705.0  (alpha = 0.01)

Used for Table H ==>     R (# groups) =   7,    df (# reps-1) =       2
Actual values    ==>     R (# groups) =   7,    df (# avg reps-1) =   2.00

--------------------------------------------------------------------------------

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal
      but do not differ greatly, the Hartley test may still be used
      as an approximate test (average df are used).
```

BAS 781 02 H & L. gibba 7-day biomass (ug/L
File: 0404b Transform: NO TRANSFORMATION

Bartletts test for homogeneity of variance

Calculated B statistic = 5.75
Table Chi-square value = 16.81 (alpha = 0.01)
Table Chi-square value = 12.59 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 2.00
Used for Chi-square table value ==> df (#groups-1) = 6

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

BAS 781 02 H & L. gibba 7-day biomass (ug/L
File: 0404b Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	488.023	81.337	66.181
Within (Error)	14	17.200	1.229	
Total	20	505.223		

Critical F value = 2.85 (0.05,6,14)
Since F > Critical F REJECT Ho:All groups equal

BAS 781 02 H & L. gibba 7-day biomass (ug/L
File: 0404b Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	Neg control	18.600	18.600		
2	0.41	17.533	17.533	1.178	
3	1.2	18.233	18.233	0.405	
4	3.7	15.067	15.067	3.904	*
5	11	10.933	10.933	8.470	*
6	33	7.137	7.137	12.664	*
7	100	6.537	6.537	13.327	*

Dunnett table value = 2.53 (1 Tailed Value, P=0.05, df=14,6)

BAS 781 02 H & L. gibba 7-day biomass (ug/L

File: 0404b Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	Neg control	3			
2	0.41	3	2.290	12.3	1.067
3	1.2	3	2.290	12.3	0.367
4	3.7	3	2.290	12.3	3.533
5	11	3	2.290	12.3	7.667
6	33	3	2.290	12.3	11.463
7	100	3	2.290	12.3	12.063

BAS 781 02 H & L. gibba 7-day biomass (ug/L
File: 0404b Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Neg control	3	18.600	18.600	18.600
2	0.41	3	17.533	17.533	17.883
3	1.2	3	18.233	18.233	17.883
4	3.7	3	15.067	15.067	15.067
5	11	3	10.933	10.933	10.933
6	33	3	7.137	7.137	7.137
7	100	3	6.537	6.537	6.537

BAS 781 02 H & L. gibba 7-day biomass (ug/L
File: 0404b Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Neg control	18.600				
0.41	17.883	0.792		1.76	k= 1, v=14
1.2	17.883	0.792		1.85	k= 2, v=14
3.7	15.067	3.904	*	1.88	k= 3, v=14
11	10.933	8.471	*	1.89	k= 4, v=14
33	7.137	12.666	*	1.90	k= 5, v=14
100	6.537	13.329	*	1.91	k= 6, v=14

s = 1.108
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds	Std.Err.	Lower Bound

		Lower	Upper		/Estimate
EC5	0.27	0.058	1.2	0.32	0.22
EC10	0.72	0.20	2.6	0.26	0.28
EC25	3.7	1.5	8.8	0.18	0.42
EC50	23.	14.	37.	0.10	0.61

Slope = 0.854 Std.Err. = 0.109

!!!Poor fit: p = 0.0043 based on DF= 4.0 14.

LEMNABIO.TXT : BAS 781 02 H & L. gibba 7-day biomass (ug/L

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	3.00	18.6	19.3	-0.651	100.	0.00
0.410	3.00	17.5	17.9	-0.402	93.2	6.84
1.20	3.00	18.2	16.6	1.64	86.2	13.8
3.70	3.00	15.1	14.4	0.641	74.9	25.1
11.0	3.00	10.9	11.7	-0.729	60.6	39.4
33.0	3.00	7.14	8.56	-1.43	44.5	55.5
100.	3.00	6.54	5.61	0.932	29.1	70.9

!!!Warning: EC5 not bracketed by doses evaluated.